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Gel Filtration with Buffers Containing Dextran

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It has been shown,[1,2] that the solubility of a number of proteins decreases in dextran solutions ($M_w > 150\,000$). This decrease is dependent on the molecular size of the protein and the concentration of dextran. It was explained as a steric exclusion of the proteins from the polysaccharide solution.

By gel filtration[3] substances are separated according to differences in molecular size. The gel can be regarded as a three-dimensional network of straight polymer fibres and the gel filtration process can be explained as a steric exclusion of solutes from this network.[4] Laurent and Killander showed that there is good agreement between the available volume for certain proteins in a dextran solution and a dextran gel of the same concentrations.[4] They concluded that the cross-linking in the gel does not have any essential influence on the exclusion property.

If a polymer is added to the buffer used in gel filtration experiments, it should have exclusion properties similar to the polymer gel. The available volume for a substance in the buffer phase should decrease as a results of the exclusion by the polymer solution and the partition coefficient between the gel and liquid phase should increase. Thus the elution volume for a substance should change.

The purpose of this investigation was to examine the effect on the elution volume of a protein in gel filtration when the buffer solution contains a polymer.

The chromatographic tube was made of plexiglass and had adjustable plungers with porous membranes of polyethylene in the ends. After the tube had been packed with Sephadex G-200 (Pharmacia, Uppsala, Sweden) the plungers were adjusted to the size of the column (2 × 108 cm). A peristaltic pump was connected to the lower end of the tube giving a reverse flow. The transmission of the eluate at 254 mμ was continuously registered in a 0.3 cm cuvette with an Uvicord absorptiometer (LKB, Stockholm, Sweden) connected to a recording potentiometer. The flow rate was approximately 3 ml/cm²/h with 0.05 M phosphate buffer, pH 7.4, containing 0.1 M sodium chloride. To the buffer was added Dextran 150 (weight average molecular weight 153 000, Pharmacia, Uppsala, Sweden) to the final concentrations of 1 or 2 % (w/v). The samples were applied in 1.0 ml volume. After each sample a small volume of 5 % (w/v) sucrose in the corresponding buffer-dextran solution followed. The eluate was collected in small test tubes with a fraction collector. The content in the tubes was weighed and the volume was calculated. As partial specific volume for dextran the value 0.61 was used.[5] The optical density at 280 mμ in a 1.0 cuvette was measured with a Beckman DU spectrophotometer.

The void volume of the column was determined with 2.5 mg Blue Dextran 2,000 (average molecular weight 2 000 000, Pharmacia, Uppsala, Sweden). It was redetermined for each concentration of dextran used in the eluent. As test substance human serum albumin (AB Kabi, Stockholm, Sweden) was chosen. This albumin (55 mg) was separated into four fractions. The first three consisted of the polymer, dimer, and monomer fraction of albumin.[6] The fourth fraction was of uncertain origin. Probably it consisted of a bacteriostatic agent added to the protein by the manufacturer. It was shown that this fraction and tritiated water emerge at the same place in the elution diagram. The elution volume of the fourth fraction was considered to correspond to the total volume of the column, which was in good agreement with direct measurements on the tube after the gel had been removed.

The polymer fraction of albumin was eluted with the void volume. When the dextran content in the buffer solution was increased, the void volume increased indicating that the gel had shrunk owing to the increased osmotic pressure in the buffer-dextran solution. The dimer and monomer fraction of albumin were also eluted later, the higher the concentration of dextran in the buffer. On the contrary the fourth peak emerged with the same volume independent of the dextran concentration.

The different values of the void volume and the elution volume were used for calculations. The mean value of the elution volume of the fourth fraction in seven runs was used as total volume.

As demonstrated in Fig. 1 the presence of dextran in the buffer has a great influence on the elution volume of albumin. When no dextran is present in the buffer the partition coefficient, K_{av},[4] for the monomer fraction of albumin is 0.42, which is in good agreement with the values found by others.[4] With 1 and 2 % dextran in the buffer the corresponding K_{av} values are 0.52 and 0.58. In a separate experiment using another column packed with a different batch of Sephadex G-200 the corresponding K_{av} value was 0.72, when 4 % Dextran 150 was added to the buffer.

The results suggest that gel filtration can be used to determine the exclusion properties of the polymer solutions used as eluents. It is also possible that this modification of gel filtration can be used for increased resolution in separation work, for instance by using dextran gradients. This investigation will be described in detail in a later publication.

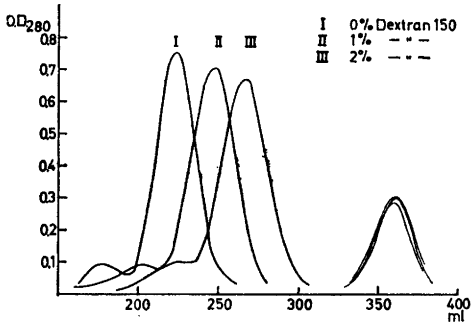

Fig. 1. 55 mg of human serum albumin chromatographed on a column of Sephadex G-200 with various concentrations of Dextran 150 in the eluent. The first eluted peak ("albumin polymers") is not shown in the diagram.

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The Constituents of Conifer Needles

III.* Isolation of β-D-Glucosides of Guaiacyl Glycerol from *Pinus silvestris* L.

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Further studies on the low-molecular weight components of the water-soluble fraction of an ethanol or acetone extract of *Pinus silvestris* L. needles [1] (collected in the autumn) have resulted in the isolation of shikimic acid, sequoitol, L-rhamnose, D-mannitol and two new glucosides.

Carbon column chromatography using gradient elution with aqueous ethanol separated the two glucosides. Higher oligosaccharides and other compounds present in the glucoside-containing fractions were removed by chromatography on cellulose columns. Final purification was accomplished by paper chromatography.

One glucoside (I), isolated in about 0.1 % yield of the water-soluble compo-

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